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# Botulinum Toxin Fact Sheet[*]

## I. Introduction

The botulinum toxins as a group of toxins are among the most toxic compounds known to man. They are lethal neurotoxins with effects that are irreversible. A dose of 1 µg may be fatal if swallowed or inhaled. The botulinum toxin is 15,000 times more toxic than the VX nerve gas, and 10,000 times more toxic than the Sarin nerve gas. (1)

The botulinum toxins are a group of heat liable neurotoxin of 150,000 Da molecular weight, produced by the anaerobic gram-positive bacterium, ***Clostridium botulinum***. The toxins are composed of two chains—a heavy chain of 100,000 Da and a light chain of 50,000 Da.

- The heavy chain is required for binding of the toxin to the presynaptic nerve terminal at the neuromuscular junction and at cholinergic autonomic sites. The carboxy-terminal part of the heavy chain ($H_c$) is a specific portion of the heavy chain that is responsible for the neuro-specific binding.

- The light chain is required for intracellular catalytic activity. The binding and internalization prevents the release of acetylcholine, thus blocking neurotransmission and causing paralysis.

The fragments themselves are <u>not toxic</u>. The heavy chain will bind and initialize the internalization process. The light chain will then initiate intracellular catalytic activity. Symptoms will vary depending on what form of the disease is present. The target organs are the cholinergic nerve endings throughout the body. The material acts principally by paralyzing or blocking the neuro-transmission of signal or synapses of the peripheral nerves in the muscle. This ultimately leads to respiratory failure followed by death.

There are three forms of botulism:

- <u>Classical Botulism</u> is a severe intoxication resulting from the ingestion of contaminated food. This illness is characterized by clinical manifestation related to the central nervous system. Fever is usually absent unless accompanied by an infection, but the initial symptoms may include visual difficulty such as: blurred vision, double vision, photophobia, dizziness or vertigo. Slurred speech, nausea, or vomiting may follow. Prior to 1950, the fatality rate was 60%(1): Today with good supportive care and use of anti-toxins, the case fatality rate has been reduced to 15%. Recovery is slow taking up to many months to recover (2).

- <u>Wound botulism</u> yields the same clinical picture after a wound has been contaminated with spores. Infection occurs with subsequent germination, multiplication of organisms, and elaboration of toxin.

· Infant botulism is the most common form of botulism that results from the ingestion of contaminated foods such as milk. Once ingested, the intestines become contaminated to produce the toxin. Infant botulism has a wide spectrum of clinical severity ranging from mild illness to sudden infant death. The case fatality rate in the U.S. is about 2% (2) .

## II. HAZARDS AND RISKS

***Clostridium botulinum***. Is classified as a Risk Group 2 agent (where therapeutic intervention is available) by both the National Institutes of Health (NIH)[3], and the Centers of Disease and Prevention (CDC)[4]. The United States government has also declared this agent as a "Select Agent" with the CDC and USDA. [5][6].

As seen in the table below, the bacterium produces a number of heat liable neurotoxins.

Table 1: Characteristics of Classical Botulinum Toxin[7]

| Type | Host | Source | Exposure Routes | | | LD 50 |
|---|---|---|---|---|---|---|
| | | | Inhalation | Ingestion | Skin | (ug/kg) |
| A | Human * | Infant Botulism | 1-5 days | 12-24 hrs | X | 0.0012 |
| B1 | Human * | Infant Botulism | 1-5 days | 12-24 hrs | | 0.002 |
| C | Bird/Mammals | | X | X | | 0.011 |
| C2 | Bird/Mammals | | X | X | | 0.0012 |
| D | Bird/Mammals | | X | X | | 0.0004 |
| E | Human * | Seafood Marine Mammals | X | 12-24 hrs | | 0.0011 |
| F | Human | | 12-36 hrs | 12-24 hrs | | 0.0025 |
| G | Human (?) | | 12-36 hrs | 12-24 hrs | | No data |

(*) - Most Commonly found

Documents from USAMRIID [8] and the Defense Intelligence Agency[9] have determined that the botulinum toxin would make an effective biological agent. As seen in Table 2, approximately 8.0 kilograms of material would be required to exposure a 100 square kilometer area under ideal meteorological conditions. The botulinum toxin could be used as a biological warfare weapon as it spreads into the air, or through contaminated food or drink.

When inhaled, these toxins produce a clinical picture very similar to food-borne intoxication, although the time to onset of paralytic symptoms may actually be longer than for food-borne cases.

Table 2:Quantity of Biological or Chemical Agent Required for a 100 Sq Ft Area [8][9]

| | Type | Agent (Disease) | Quantity Required |
|---|---|---|---|
| | | | (Kilograms) |
| 1 | Biological Agent | Francisella tularensis (Tularemia) | 0.2 |
| 2 | Biological Agent | Bacillus anthracis (Anthrax) | 0.2 |
| 3 | Biological Material | Botulinum Toxin | 8.0 |
| 4 | Biological Material | Ricin | 8,000 |
| 5 | Biological Material | Aflatoxin | 8,000,000 (8,000 Metric Tons) |
| 6 | Chemical Agent | Sarin | 100,000 (100 Metric Tons) |

# III. RECOMMENDATIONS

It is important for all personnel working with such materials to be advised of the potential mode of transmission, and to use the proper precautions listed below. The following recommended work practices listed below may be more or less conservative than those practiced at other facilities.

Work Practices:

- General Work Practices: A Biosafety Level 2 containment level using biosafety level 2 work practices are required for handling, and use of non-aerosolize concentrations of research amount (less than 10 liters) of this material in research facilities [4].

  Work with this neurotoxin should include the following work practices.

  - Inspect the containers for damage during shipment. If damaged contact the vendor immediately, and decontaminate and dispose of the shipment immediately. If the vials were not damaged excess packing materials may be disposed in the regular trash.
  - Log in the select agent shipment on the select agent inventory sheet.
  - All packages containing select agents should be opened in a fume hood, glove box or ducted biosafety cabinet.
  - All high-risk operations should be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
  - If the botulinum toxin is received as a powder, place a pad on the inside of the fume hood, glove box or ducted biosafety cabinet in order to minimize the spread of contamination during the weighing process. Ensure the fume hood, glove box, or ducted biosafety cabinet is working properly with an inward airflow prior to initiating work. All work should be performed within the operationally effective zone of the fume hood or biosafety cabinet.

- When handling dry forms of the powder that are electrostatic, do not wear gloves (such as latex) that generate static electricity. Use a glove bag within a fume hood or biological safety cabinet, a glove box or class III biosafety cabinet.
- If you are aliquoting samples, place an absorbent pad on the bottom of the fume hood or ducted biosafety cabinet in order to contain any potential spills. Ensure the fume hood, glove box, or ducted Biosafety cabinet is working properly with an inward airflow prior to initiating work. All work should be performed within the operationally effective zone of the fume hood or biosafety cabinet. Resuspend the toxin by extremely careful and slow titration, rinsing down the walls of the tube in the process. Avoiding foaming and aerosolization. Aliquot 5 mls of 10% bleach solution into a 50 ml conical tube and place the open tube in the tube rack. This tube will serve as a waste receptacle for contaminated pipette tips. When vacuum lines are used with systems containing toxins, they should be protected by a HEPA filter to prevent entry of the toxin into the lines. Sink drains should be similarly protected when water aspirators are used.
- Place the toxin vials in an unbreakable, clean secondary storage container and transfer to storage at the appropriate temperature. Decontaminate the exterior surfaces of all materials leaving the biosafety cabinet, including the closed aliquot tubes with a 10% household bleach solution.
- Until the fume hood, glove box or ducted biosafety cabinet is decontaminated, the equipment should be posted to indicate that toxins are in use, and access to the equipment and apparatus is restricted to authorized personnel.
- Toxins should be transported only in leak-proof secondary containers.
- Standard sharps must not be used with botulinum toxin unless specifically approved by Environmental Health and Safety. If it is absolutely necessary to use sharps with botulinum toxin, sharps with engineering controls must be used.
- If a spill occurs outside of the fume hood or ducted biosafety cabinet, notify lab personnel and evacuate immediately. Close the doors as you exit to allow the aerosols to settle. Notify EH&S immediately! Do not re-enter the facility.
- Personnel decontaminated the spill must wear the minimum PPE of safety goggles, disposable lab coat, double gloves and shoe coverings. Respiratory protection may be necessary based on a risk assessment of materials, concentration, and amounts used.
- Cover the spill area with absorbent material from the outside in and cover with 10% household bleach solution for a 30-minute contact time. Do not splash the material in your face, and attempt to limit exposure. Clean up the material and place in double red biohazard bags. Ventilate the area and wash the spill site again with soap and

water after decontamination is complete. Notify your supervisor and EH&S of the spill immediately.

- Medical Surveillance: Enrollment into the Medical Surveillance Program at your facility is mandatory. In accordance with a NIH document, "Laboratory Safety Monograph, a supplement to the Guidelines for recombinant DNA research" dated July 1978 [13]:

    - Immunization is generally recommended for people at risk (i.e., laboratory workers) who will be engaged in research with infectious agents for which an effective vaccine is available. Currently, there is no safe vaccine available for this toxin.
    - A penta-valent (A,B,C,D,E) toxoid is available under IND status and has been found effective against aerosol challenges. It is a formalin inactivated product and may not be available due to its IND status, cost and limited supply. The toxoid is administered at 0, 2, and 12 weeks with an annual booster. (BLUE BK)
    - Studies have also shown that there are no known benefits when given prophylactically following an exposure.

- Decontamination: Soap and water for surfaces and equipment is recommended for general cleaning.

    - A concentration of 0.5 ppm (5mg/L) of sodium hypochlorite is recommended for decontamination.
    - Work surfaces can also be decontaminated with a 2.5% sodium hypochlorite, with or without 0.25 N sodium hydroxide for 30 minutes. (USAMRIID)
    - Dry waste must be placed in double red biohazard bags autoclaved in an autoclave approved for Medical Waste treatment at 121 °C for 60 minutes at 18 psi.
    - Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair.
    - If decontamination is not possible/practical, materials (e.g. used gloves) should be disposed of as hazardous waste. Materials contaminated with infectious agents as well as toxins should be also be autoclaved or rendered non-infectious before leaving the laboratory.
    - Ultra-violet light has no effect on the botulinum toxin.

Engineering Controls

- Engineering controls are used to isolate or remove hazards from the workplace in order to reduce the potential for exposure. Engineering controls in combination with safe work practices that alter the manner in which tasks are performed are expected to be primary means of eliminating or further minimizing the risk of occupational exposures. The use of specific engineering controls will vary and typically are:

- mechanical aids (e.g., tongs, tweezers),
- dead air boxes,
- sharp containers,
- laboratory-type fume hoods: must have visual indication of airflow or alarmed to indicate whenever airflow has fallen below acceptable standards.
- biological safety cabinets (BSC): must be certified at the time of installation, when moved or relocated, and annually thereafter,
- shielding,
- containment protection for vacuum systems,
- safety centrifuge cups, and
- special shipping containers for transporting biological materials and animals
- Placarding: When toxins are in use, the room should be posted to indicate "Toxins in Use-Authorized Personnel Only". Any special entry requirements should be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while toxins are in use.

· Examples of facility design controls are depend on the risk of transmission of specific biohazardous agents.

- high efficiency particulate air (HEPA) filters,
- interlock systems, and
- negative airflow units.
- Access Control: Access to areas containing toxins should be restricted to those whose work assignments require access. Principal Investigators are responsible for ensuring that all laboratory workers and visitors understand security requirements and are trained and equipped to follow established procedures.
- Lockable Equipment: Freezers, refrigerators, cabinets, and other containers where stocks of select agents are stored must be locked when they are not in direct view of the workers (e.g., when located in unattended storage areas).

Personal Protective Equipment (PPE):

· Identifying and understanding the hazard and then matching the needed PPE to the workplace hazard is the key to selecting effective and appropriate protection. Personal protective equipment is to be used only as supplemental protection if there is still a residual risk of exposure after engineered and administrative controls are implemented, then PPE may be needed.

When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic by dermal exposure), select gloves that are known to be impervious to the toxin. Consider both the toxin and the diluent when selecting gloves or other protective clothing. If infectious agents and toxins are used in an experimental system, consider both when selecting protective clothing and equipment. Respiratory protection may be required if aerosols may be generated and it is not possible to use containment equipment or other engineering controls. Examples of PPE are:

- Gloves: non-permeable nit rile or latex gloves,
- Coats: disposable long-sleeved laboratory coats
- Gowns: optional
- Safety glasses or goggles

## IV REFERENCES

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